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Houghton
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Date:



**PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re the Application of

Group Art Unit: 1642

JC Xu et al.

Application No.: 09/232,880

Filed: January 15, 1999

For: COMPOSITIONS FOR IMMUNODIAGNOSIS OF PROSTATE
CANCER AND METHODS FOR THEIR USE

Examiner: Alana Harris, Ph.D.

Docket No.: 210121.428c6

**DECLARATION UNDER 37 C.F.R. §1.132 OF
RAYMOND L. HOUGHTON, PH.D.**

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D. C. 20231

The undersigned, Dr. Raymond L. Houghton, hereby declares:

1. I am a Senior Scientist employed at Corixa Corporation, the assignee of the subject patent application.

2. The following studies were carried out under my direct supervision.

Quantitative real-time PCR for P703P, F1-12 (also known as P504S) and P710P was performed either on an Applied Biosystems 7700 Prism or on a GeneAmp 5700 Sequence Detection System (PE Biosystems, Foster City, CA). The 5700 system uses SYBR green, a fluorescent dye that only binds to double stranded DNA. Matching primers and fluorescent probes were designed for each of the genes according to the

primer express program (PE Biosystems). Primers and probes so produced were used in the universal thermal cycling program in real-time PCR. They were titrated to determine the optimal concentrations using a checkerboard approach with a pool of cDNA from target tumors. The reaction was performed in 25 μ l. The final probe concentration in all cases was 160 nM. dATP, dCTP and dGTP were at 0.2 mM and dUTP at 0.4 mM. Amplitaq gold and Amperase UNG (PE Biosystems) were used at 0.625 units and 0.25 units per reaction. $MgCl_2$ was at a final concentration of 5 mM. Trace amounts of glycerol, gelatin and Tween 20 were added to stabilize the reaction. Each reaction contained 2 μ l of diluted template. The cDNA was diluted 1:10 for each gene of interest and 1:100 for β -actin. Primers and probes for β -actin (PE Biosystems) were used in a similar manner to quantitate the presence of β -actin in the samples. In the case of the SYBR green assay, the reaction mix (25 μ l) included 2.5 μ l of SYBR green buffer, 2 μ l of cDNA template and 2.5 μ l each of the forward and reverse primers for the gene of interest. This mix also contained 3 mM $MgCl_2$, 0.25 units of AmpErase UNG, 0.625 units of Amplitaq gold, 0.08% glycerol, 0.05% gelatin, 0.0001% Tween 20 and 1 mM dNTP mix. In both formats, 40 cycles of amplification were performed.

In order to quantitate the amount of specific cDNA (and hence initial mRNA) in the sample, a standard curve is generated for each run using the plasmid containing the gene of interest. Standard curves were generated using the Ct values determined in real-time PCR which were related to the initial cDNA concentration used in the assay. Standard dilutions ranging from $20\text{-}2 \times 10^6$ copies of the gene of interest were used for this purpose. In addition, a standard curve was generated for the housekeeping gene β -actin ranging from 200fg-2000pg to enable normalization to a constant amount of β -actin. This allowed the evaluation of the over-expression levels seen with each of the genes.

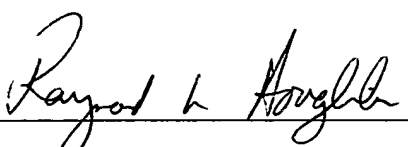
An extensive panel of tumors and normal tissues was used to determine the specificity of the genes of interest. This panel comprised 22 prostate tumors, 2 metastases, 3 benign prostatic hyperplasia (BPH), 3 normal prostate and 32 other normal tissues. Table 1 summarizes the expression of the different genes in normal prostate, prostate tumors and metastases.

Table 1

Gene	Normal prostate	BPH	Prostate tumor	Metastases	Expression profile
P504S	0/3	0/3	9/22	2/2	Liver, salivary gland, skeletal muscle
P703P	3/3	3/3	20/22	1/2	Clean normal tissue profile
P710P	0/3	0/3	10/22	1/2	Clean normal tissue profile

The genes P504S, P703P and P710P were further evaluated using real-time PCR on a panel of tissue that included 6 normal prostate samples, 6 BPH samples, 22 prostate tumors ranging in Gleason grade from 4-10, and 2 metastases. All three genes appeared to increase in expression level with increasing Gleason score. For comparison, expression of prostate specific antigen (PSA) was essentially independent of Gleason score.

3. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.



Raymond L. Houghton

11/6/00
Date